1D and 2D Quantification Methods

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Metabolite concentrations ([M]) can be estimated from MR spectra, because they are proportional to the areas of the signals produced (S_M).

$$S_M \propto [M] \times VOI \times N_{av} \times N_p \qquad\qquad [1]$$

where VOI is the volume of interest excited, N_{av} is the number of averages, and N_p is the number of atoms contributing to the signal (e.g. 3 for the protons in a CH_3 group).

The proportionality depends upon a number of factors. Some of these can be assumed to be invariant between different examinations at the same site, such as the field strength B_0 and various coil characteristics. Other factors vary, but can be corrected for easily: e.g. if the receive gain is higher by 1 unit, then the signal will be doubled (if that is how the receiver gain scales on a given machine). Still other factors vary both between and within subjects, and may be more difficult to account for in absolute quantification. These will be described below.

This talk will outline the steps involved in analyzing single voxel spectroscopy (SVS) and magnetic resonance spectroscopic imaging (MRSI) datasets, along with the problems encountered and some possible solutions.

1. Estimate the metabolite peak areas.

Peak areas can be found most simply by integration. This may be adequate for isotopes other than proton, and for ^{1}H-MRS at long echo times. However, for ^{1}H-MRS at echo times below about 100 ms it is not a very specific or reproducible technique, because the metabolite signals of interest overlap with signals from macromolecules, residual water, and other small metabolites.

Preferably, automated spectral fitting routines can be applied. These methods, whether applied in the time domain (e.g. MRUI: http://www.mrui.uab.es) or the frequency domain (e.g. LCModel: Provencher 1993), work by fitting the observed signals against a user-input model of the metabolites thought to be present. These spectral libraries can be either simulated (Young et al, 1998) or determined experimentally for a given echo time. Macromolecule signal can also be included in frequency-domain models (Seeger et al, 2003); and in time-domain modelling, manipulation of the initial data points can reduce their contribution. However, the difficulty in discriminating between these broad signals, the overlying small metabolite signals, and the baseline, remains the main source of variability in fitting of short echo time data.

The application of spectral fitting routines to MRSI data can be time-consuming, but not unfeasible on modern computers. Currently we use free queuing software (Sun N1 Grid Engine 6: Sun Microsystems Inc, Santa Clara, CA, USA) to share LCModel jobs between 3 licensed UNIX machines, for a total processing time of about 10 minutes for a 2D dataset of 50-70 spectra.

Peak fitting of MRSI data is often more challenging than for SVS. Although the data quality is not inherently worse, the optimization of magnetic field homogeneity (shimming) over large volumes of tissue is often less successful, which causes some undesirable effects. The variation in B_0 over the VOI causes relative frequency shifts between voxels, which must be corrected prior to peak fitting. Variation in B_0 within a voxel causes signals to broaden, which makes overlapping peaks harder to resolve, and in extreme cases signal is lost entirely. Variation in the frequency and width of the water peak causes variability in the efficiency of the water suppression pulses: typically the water peak may be over-suppressed in some voxels while it is under-suppressed in others, leading to differences in fitting of the baseline.

When using automated spectral fitting routines, it is important to evaluate the quality of the fits. However, examining a printout for each voxel in a large MRSI dataset would be laborious. Instead, one possibility is to examine maps of the metabolite concentrations and of the Cramer-Rao bounds, to identify any danger spots. Spectral fits from such voxels should be examined, along with a random selection of spectra from other locations. Isolated voxels with bad fits might be salvageable, e.g. by shifting the frequency and re-running the fitting. For more widespread or severe problems, datasets might need to be rejected.

2. Calibrate the peak areas, to estimate metabolite concentrations or ratios.

As mentioned in the introduction, the absolute scaling factor to convert signal area to concentration is difficult (if not impossible) to determine. Instead, we estimate concentration with reference to some known or assumed standard (Ref):

$$[M] = [Ref] \text{ x } S_M/S_{Ref} \qquad\qquad [2]$$

The simplest way to calibrate peak areas is internally, by taking the ratio between a metabolite of interest, e.g. NAA, and a reference peak, usually either creatine or water. A concentration can be calculated if the reference is assumed constant: typically a value of 10 mM might be assumed for creatine, or about 55 M for water. An additional correction factor for the number of atoms may be necessary if this differs between the metabolite of interest and the reference ($N_p = 3$ for creatine, 2 for water).

However, realization is growing that the content of creatine and water may not be constant, either in different tissues in the brain (Hetherington et al, 1994; McLean et al, 2000) or in disease (Helms, 2001; Vrenken et al, 2005). The use of water as a reference has additional drawbacks for MRSI: either an additional MRSI dataset of unsuppressed water must be acquired, which can double the time taken, or metabolites must be analyzed in spectra without water suppression, which tends to be unreliable due to artifacts such as eddy currents and water harmonics (Clayton et al, 2001).

External calibration is also possible, where the peak areas are compared to reference spectra from a phantom scanned on either the same or a separate occasion. Corrections may be needed for differences in voxel volume, number of scans, and settings of the transmitter and receiver. The transmitter correction makes use of the reciprocity principle (Hoult & Richards, 1976): if it is harder to transmit signal into the sample, it is also harder for the signal to get back out. If the phantom data is acquired in the same session as the subject data, then corrections for coil sensitivity are generally needed, since the sample will by necessity be located distant from the most sensitive volume of the coil (Helms 2000). Alternatively, if the

inter-session reproducibility is acceptable, calibration can be performed via separate regular (e.g. weekly) phantom measurements, to account for the temporal drift in scanner performance.

Additional problems arise when using rf coils with inhomogeneous profiles, such as surface coils, and at higher field strengths. At 1.5 Tesla, using a standard birdcage head coil, typical variation of <5% in B_1 is seen over the brain (Barker et al, 2000). At 3T and above, such homogeneous signal is unattainable, due in part to dielectric resonance (Hoult, 2000). Therefore, quantification of metabolite concentrations must involve measurement of and compensation for the B_1 variation (Pan et al, 1998).

For absolute quantification, corrections also are needed for T_1 and T_2 relaxation of the metabolite signals, and of the reference signals. For LCModel, for example, full relaxation information would be needed on all the chemicals in the basis set (vitro), and T_1 and T_2 would need to be measured *in vivo*, so as to allow the calculation of corrected concentrations:

$$[M]_{cor} = [M] \times \exp(-TE/T_{2vitro})/\exp(-TE/T_{2vivo}) \times [1 - \exp(-TR/T_{1vitro})]/[1-\exp(-TR/T_{1vivo})] \quad [3]$$

Usually it is considered unfeasible to collect full relaxation data in each subject due to time constraints. Because of this, and also due to differences between different acquisition and analysis methods, reported concentrations are often not directly comparable between studies, and should be thought of in 'institutional units' rather than 'millimoles per litre'.

3. Allow for the effects of the excitation profile.

When frequency-selective pulses are used to select a VOI, such as in PRESS or STEAM, they select a different volume for each point in the spectrum. This chemical shift artifact can lead to a relative offset of more than 1cm between the volumes selected for NAA and myo-inositol in MRSI experiments. Additionally, the selected VOI is not a perfect rectilinear box: a transition band around the periphery is imperfectly excited.

One approach to make the slice profile more ideal is to use high-bandwidth pulses for excitation (Ordidge et al, 1996). Another is to excite a VOI larger than desired, and use outer volume suppression (OVS) pulses with high bandwidth to exclude the unwanted signals (Tran et al, 2000). MRSI can even be performed relying solely on OVS pulses to exclude unwanted signals; but in practice this works well only at longer echo times, when the large lipid signals from the scalp have partially decayed.

In MRSI processing, the commonest method to account for the excitation profile (and in many ways the best) is to discard data from those rows and columns of the grid near the outer edge of the VOI. If this is impossible (e.g. the lesion of interest is close to the scalp and therefore to the edge of the VOI), it may be possible to correct for the slice profile during processing. The shape of the VOI can be determined either experimentally or through simulations, and this can be used to differentially weight singlet peaks at different frequencies in the peripheral voxels (Wild & Marshall, 1997, McLean et al, 2000). However, spectra from such regions should be used with caution, as they have inherently lower SNR, and coupled peaks can be particularly badly affected by the lower flip angles (Thompson & Allen, 1999).

4. Allow for the tissue composition of voxels.

Spectroscopic voxels in the brain will typically contain a mixture of tissues: white matter, grey matter, cerebrospinal fluid (CSF), and (sometimes) lesions. The metabolite contents of these differ. CSF is presumed to contain no measurable metabolite signal, except perhaps lactate and glucose, so it is often desirable to correct for its presence (i.e., normalize to 100% brain tissue) before comparisons are made. Lesions also can have markedly different metabolic content to the surrounding tissues, so it is desirable to estimate what proportion of the voxel is composed of lesions. Finally, several studies have suggested that grey matter has a greater content of glutamate and/or glutamine, creatine, and perhaps myo-inositol than white matter, and less choline, particularly in the occipital lobes (Pan et al 1996, Noworolski et al, 1999, McLean et al, 2000). Therefore, data should be analyzed if possible using a statistical model that takes tissue composition into account.

Different methods exist for determining tissue composition: one of the easiest to apply is segmentation of structural images. We use Statistical Parametric Mapping (Ashburner & Friston, 1997) to segment T_1-weighted gradient echo images into maps of grey matter, white matter, and CSF (McLean et al, 2000). If lesions are present, then another image contrast may also be needed: e.g. in multiple sclerosis, we use dual-echo images (proton-density and T2-weighted) to determine a lesion mask, which is then applied to the tissue maps to create four tissue classes (Chard et al, 2002). The amount of each tissue in each voxel can then be estimated and output along with the metabolite concentrations for spreadsheet-based analyses.

The signal from macromolecules within the brain (visible at echo times $< c$. 100ms) may also be stronger in grey matter than white (Hofmann et al, 2001). This might not affect the quantification of the major singlet peaks, if spectral fitting is used which incorporates a compensation for macromolecules; however, glutamate and glutamine in particular are difficult to resolve from these underlying broad resonances (McLean & Barker, 2005).

Conclusion

 The only gold standard available to validate metabolite concentration estimates is *ex vivo* biochemical analysis (unfortunately, this is an unpopular procedure with human volunteer subjects). Studies on animals and on human surgical specimens suggest fair agreement between results *in vivo* and *in vitro* (Barker et al, 1994; Petroff et al, 1995). The challenge in the years ahead is to continue to improve both the precision and accuracy of our metabolite measurements.

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